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Use of Genetic Methods for Detection of *Streptococcus Pneumoniae* Virulence Factors Isolated from Patient with Pneumonia

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Abstract
This study aimed to detection of *Streptococcus pneumoniae* and detection of their virulence factors , For this purpose 60 sputum samples were collected from patients with bacterial pneumonia all patient arrived to critical care unit (CCU). Bacterial culture technique and genetic technique were applied. The result showed that *Streptococcus pneumonia* detected in rate of 10% . results of mPCR showed that the *PlyA&LytA*detected in rate of 100% while *PsaA*detected in rate of 71.4%.

Introduction

Pneumonia is an inflammation of lung parenchyma tissue. it caused by many bacterial spp. Such as *Streptococcus pneumoniae , Haemophilusinfluenzae Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Moraxella catarrhalis, Neisseria meningitides* (Welp&Bomberger, 2020)

*Streptococcus pneumoniae*belongs to the family streptococcaceae. It is a gram-positive cocci , fastidious bacteria and facultative anaerobic, but can grow aerobically, caused α-hemolytic, and sensitive to optochin test. (Weisbroth, & Kohn, 2020)

*Streptococcus pneumoniae*is one of normal nasopharyngeal microbial but may be became pathogen and caused many infection such as septicemia, meningitis, pneumonia, and mild upper respiratory infections, it can transmission by respiratory secretions that contaminated hand, instruments or airborne droplets (Aykac*et al.*,2020).

The virulence genes can be acquired by many way such as spontaneous mutations and gene exchange via horizontal gene transfer (D'Mello, 2021). And may be regulate the physical properties (adhesion, biofilm, fimbriae, and flagella or regulation of biochemical factors (enzymes, toxins or antibiotics) (Schulze et al.,2021)

Materials and methods

Samples: 60 sputum samples collected from patients with bacterial pneumonia (according to physician examination, chest X-Ray and Gram stain of sputum), all patient arrived to critical care unit (CCU) in Salahaldeen teaching hospital.
Bacterial culture : all sample cultured on Blood agar, Chocolate agar manitol salt agar, macConkey agar.

DNA extraction: DNA direct extraction from sputum by use of (Sputum DNA Isolation Kit – Morgen company -Product #: RU46100) and according to manufacturer's instructions.

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Primers used for of detection of *S.pneumoniae* : as in table (1).

Table (1): Primer used for detection of *S.pneumoniae*

ne name	Sequences		roduct size	ferences
Beta-globin		5’-GAAGAGCCAAGGACAGGTAC-3’	268	ırdain et al.,2011
		5’-CAACTTCATCCACGTTCACC-3’		

- PCR Reaction mixture and thermocyclar program used for detection of *S.pneumoniae*as in table (2) and table (3).

Table (2) PCR Reaction mixture for detection of *S.pneumoniae*

Master mix components	Amount (µM)
Master Mix	20
Forward prime (Beta-globin)	1
Reverse primer (Beta-globin)	1
DNA template	3
Nuclease Free Water	25
Total	50

Table (3): Thermocyclar program for detection of *S.pneumoniae*

Steps	Temperature (°C)	Time	Cycle
Initial Denaturation	94	5mints	1
Denaturation	94	30 seconds	35
Annealing	58	30 seconds	
Extension	72	30 seconds	
Final extension	72	7 mints	1

Detection of *S.pneumoniae*virulence factor

- Primers used for of*S.pneumoniae*virulence factors: as in table (4)

Table (4): Primers used for of*S.pneumoniae*virulence genes

e name	Sequences		roduct size	ferences
PlyA		5'-ATTTCTGTAACAGCTACCAACGA-3'	329	Salo*et al.*,1995
		5'-GAATTCCCTGTCTTTTCAAAGTC-3'		
LytA		5’-CCATTATCAACAGGTCCTACC-3	187	i et al.,2020
		5’-TAAGAACAGATTTGCCTCAAG-3’		
saA		5’-GCCCTAATAAATTGGAGGATCTAATGA-3’	14	i et al.,2020
		5’-GACCAGAAGTTGTATCTTTTTTTCCG-3’		

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- PCR Reaction mixture and thermocyclar program used for detection of *S.pneumoniae*as in table (5) and table (6).

Table (5) PCR Reaction mixture for detection of *S.pneumoniae*virulence factors

Master mix components	Amount (µM)
Master Mix	20
Forward prime *PlyA*	1
Reverse primer *PlyA*	1
Forward prime *LytA*	1
Reverse primer *LytA*	1
Forward prime (*PsaA*)	1
Reverse primer (*PsaA*)	1
DNA template	3
Nuclease Free Water	21
Total	50

Table (6): Thermocyclar program for detection of *S.pneumoniae*virulence factors

Steps	Temperature (°C)	Time	Cycle
Initial Denaturation	94	2mints	1
Denaturation	94	15 seconds	35
Annealing	58	15 seconds	
Extension	72	50 seconds	
Final extension	72	1 mints	1

Results and discussion:

From table (7) and figure (1) showed that *Staphylococcus aureus*and *Streptococcus pneumonia* diagnosed in rate of 11.6% and 7.1% respectively . while *Klebseilla pneumonia, Pseudomonas aeruginosa*and *Escherichia coli* 6.6%, 6.6% and 3.3% respectively

Table (7): results of bacterial culture and PCR test

Spp. of bacteria	Number of isolates	Isolation ratio
Staphylococcus aureus	9	15%
Streptococcus pneumonia	7	11.6%
Klebseillapneumoniae	4	6.6%
Pseudomonas aeruginosa	4	6.6%
Escherichia coli	2	3.3%

In the current study showed that *Streptococcus pneumonia* detected in rate of 10%, this results disagreed with results of (Motaweq, et al., 2015) whom recorded isolation rate 12.3% in Nagaf province

And result recorded by (Al Ghizawi et al., 2007) in basrah which are 19% for all streptococcus spp. Hassan&Majid. (2018) detection of that *Streptococcus pneumonia* in Sulaymaniyah Province in rate of 13% . Saleh, Jarullah,. (2019). Isolated of Streptococcus pneumoniae in rate of 27% Thi-Qar province.

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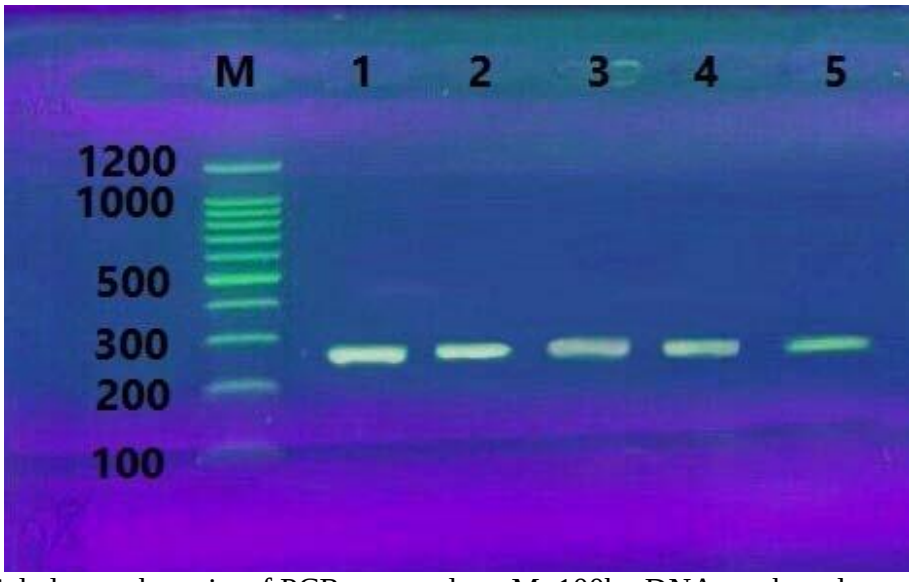

Figure (1): Gel electrophoresis of PCR out product. M: 100bp DNA marker , lanes 1-5 posative sample of *S.pneumoniae*with out product in size 268 bp fragment

From table (8) and figure (2) showed that the *PlyA&LytA*detected in rate of 100% while *PsaA*detected in rate of 71.4%

Table (8): *S.pneumoniae*virulence gene detected in current study

Gene	No of isolate	Ratio
PlyA	7	100%
LytA	7	100%
PsaA	5	71.4%

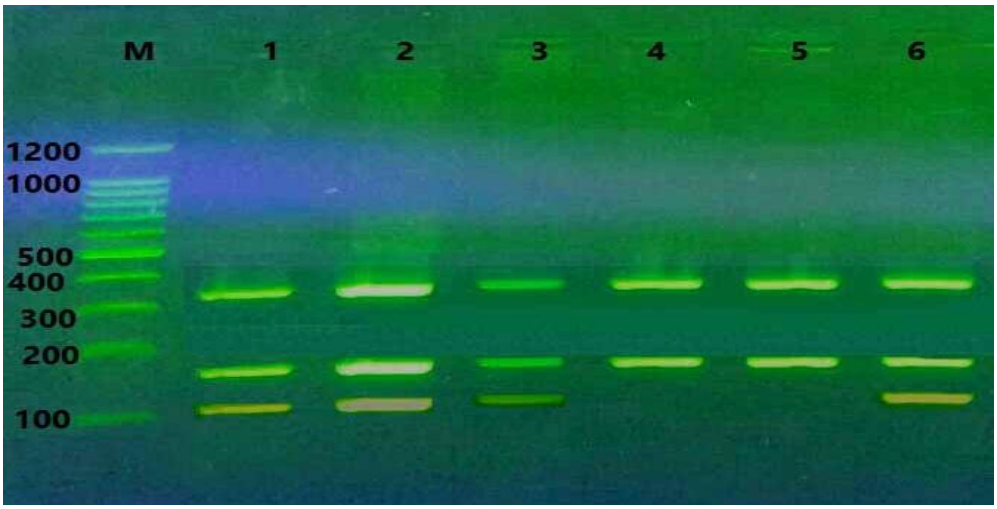

Figure (2): Result of mPCR for detection of *S.pneumoniae*virulence factors , M: 100bp DNA marker , lanes 1,2,3&6 isolates have *PlyA gene, LytA gene* and *PsaAgene* with fragment in size

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329,187 and 114 respectively. 4&5 : isolates have *PlyA gene, LytA* gene with fragment in size 329 and 114 respectively

S.pneumoniae intracellular toxin play important roles in pathogenesis. PlyA is a single chain thiol activated protein with 53kDa, consists from 471 amino acid. (Inomata et al.,2020) The main action of PlyA are autolysis of the cell (respiratory system cell as exempla), hemolysin to blood cells and decompose any eukaryotic cell containing the cholesterol (Joshi et al.,2020)

*LytA*gene detection in rate 100%, many studies detection this gene Abdeldaim*et al*.(2010) and Suzuki *et al*.(2006) with different in detection rate. *S.pneumoniae* have three autolysin enzymes which are LytA, LytB, LytC, these enzymes able to distraction of bacterial peptidoglycan and lysis the cells (Ross,2010)

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